REPRODUCIBILITY OF AN ANIMAL MODEL SIMULATING COMPLEX COMBAT-RELATED INJURY IN A MULTIPLE-INSTITUTION FORMAT

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ABSTRACT—We developed a complex combat-relevant model of abdominal and extremity trauma, hemorrhagic shock, hypothermia, and acidosis. We then simulated injury, preoperative, and operative phases. We hypothesized that this model is reproducible and useful for randomized multicenter preclinical trials. Yorkshire swine were anesthetized, intubated, and instrumented. They then underwent femur fracture, 60% total blood volume hemorrhage, a 30-min shock period, induced hypothermia to 33°C, and hemorrhage volume replacement with 3:1 isotonic sodium chloride solution (NS) at each of three centers. Hemodynamic parameters were measured continuously. Thromboelastography, arterial blood gas, and laboratory values were collected at baseline, after the shock period, and after NS replacement. Thirty-seven animals were used for model development. Eight (21%) died before completion of the study period. Twenty-nine survivors were included in the analysis. MAP (±SEM) after the shock period was 32 ± 2 mmHg and was similar between centers ($P = 0.4$). Mean pH, base deficit, and lactate levels were 7.29 ± 0.02, 8.20 ± 0.65 mmol/L, and 5.29 ± 0.44 mmol/L, respectively, after NS replacement. These were similar between centers ($P > 0.05$). Prothrombin time values increased significantly over time at all centers, reflecting a progressive coagulopathy ($P < 0.02$). Thromboelastography maximum amplitude values were similar among centers ($P > 0.05$) and demonstrated progressively weakened platelet interaction over time ($P < 0.03$). Hematocrit was similar after controlled hemorrhage ($P = 0.15$) and dilution ($P = 0.9$). The pH, lactate, base deficit, and coagulation tests reflect a severely injured state. A complex porcine model of polytrauma and shock can be used for multi-institutional study with excellent reproducibility. A consistent severe injury profile was achieved, after which experimental interventions can be applied. This is the first report of a reproducible multicenter trauma and resuscitation–related animal model.

KEYWORDS—Resuscitation, trauma, preclinical trial, shock, femur fracture, multicenter, swine, coagulopathy

INTRODUCTION

Exsanguinating hemorrhage is second only to central nervous system injury as a cause of death after civilian trauma, being responsible for up to 40% of all trauma deaths (1, 2). Conversely, hemorrhagic death accounts for most of potentially preventable deaths on the current battlefield (3). Traditional massive transfusion strategies are based on a high ratio of packed red blood cells (PRBCs) to platelets and fresh frozen plasma (FFP). Examples include recommendations of four units of PRBC per one unit of FFP (4), infusion of one to two units of FFP for each 5 units of PRBC (5), or infusion of FFP after a blood loss equivalent to one blood volume, or approximately 10 units of PRBCs (6–8).

Strategies like these contribute to the classic "bloody vicious cycle" of hemorrhage in trauma, consisting of hypothermia, acidosis, and coagulopathy. The existence of a primary coagulopathy of trauma is generally accepted, which is worsened by current fluid replacement strategies (9, 10). Coagulopathy has been shown to be an independent predictor of death in severely injured trauma patients (10). Based on observations such as these,

several authors have recently published data recommending new ratios of blood products and decreased use of crystalloid (11–15). These authors recommend the use of a 1:1 or 1.5:1 ratio of PRBC to FFP to minimize acute traumatic coagulopathy.

Combat resuscitation reflects differences between military and civilian settings. Hemorrhage is still the leading cause of death among military casualties. Injuries are most often penetrating, and massive tissue loss caused by high-energy mechanisms, often improvised explosive devices, is frequent. These factors contribute to a higher mortality rate for patients in shock—65% vs. 50% in civilian centers (16).

Regardless of the setting, massive transfusion is an uncommon event that does not lend itself well to randomized prospective controlled trials (14). Clearly, research is still needed in an ongoing effort to address several unanswered questions in massive transfusion and combat resuscitation. These include elucidating the effects of resuscitation fluids in trauma, and the search for better fluids based on minimizing the deleterious effects of resuscitation and the optimal ratios of blood products in massive transfusion.

Animal models have proven to be invaluable in investigating these questions. In addition to providing a source of data in preclinical models, they provide data in situations where human data are either scarce or is unethical to generate in experimental settings. For example, it is estimated that only 1% to 2% of civilian trauma and 7% of combat casualties require massive

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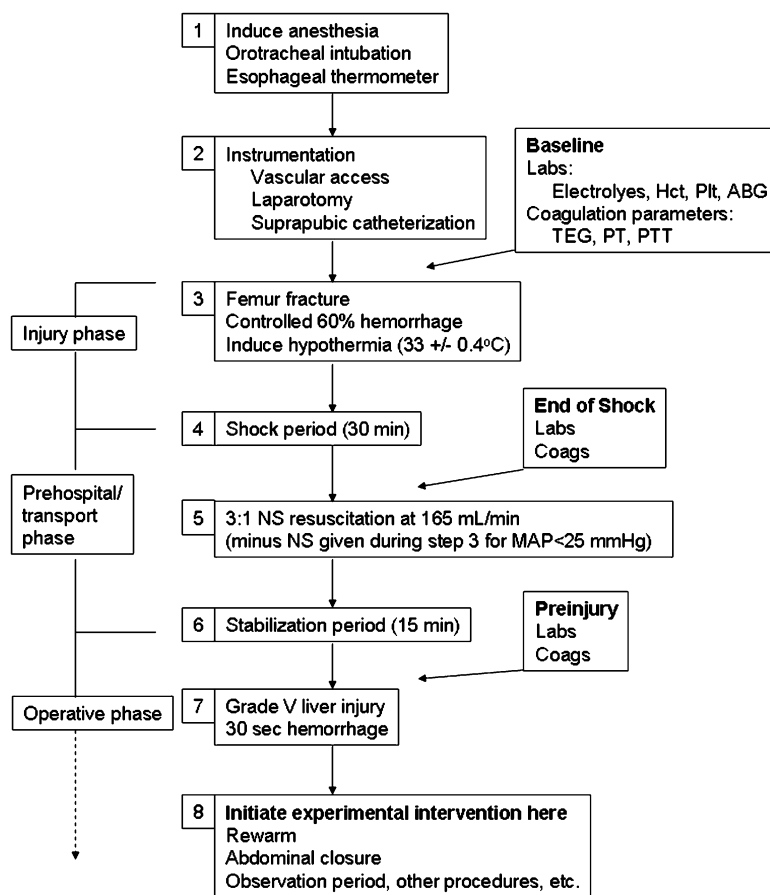| 16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT **UU**	18. NUMBER OF PAGES **10**	19a. NAME OF RESPONSIBLE PERSON
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Fig. 1. **Complex multisystem injury model with soft tissue injury, long bone fracture, and intra-abdominal injury**. Controlled and uncontrolled hemorrhage, potential for rebleeding, and the lethal triad of trauma (hypothermia, coagulopathy, and acidosis) are all components of this model. Clinically relevant phases of patient care are simulated as well. Hct–hematocrit, Labs–laboratory parameters, Plt–platelet count.

transfusion (14, 17). The relative infrequency of occurrence makes this population difficult to study, supporting the importance of animal models. For example, animal studies have shown the benefit of 3% hypertonic saline with Dextran in an uncontrolled hemorrhagic shock model (18), an increase in inflammatory gene transcription after fluid resuscitation (19), and an increase in blood loss and dilutional coagulopathy with isotonic sodium chloride solution versus Ringer's lactate solution (LR) resuscitation (20, 21).

Given the use of animal models in resuscitation research, there are inherent problems that are well recognized. A recent review of animal models for resuscitation research, particularly in the combat setting, emphasized the lack of reproducibility of results from one laboratory to another, as well as suboptimal simulation of clinical settings (22). This review outlines an urgent need for relevant models with which to test new theories and products in the mass casualty and combat realms, given both changing philosophies in resuscitation and the uniquely resource-constrained environment of the modern battlefield. A summary of the recommendations of the Military Medicine Workshop on Animal Models in Hemorrhage and Resuscitation Research in 2000 was outlined. Key variables identified related to the physiologic state of the animal, the experimental procedures, resuscitation protocol, time of end point assessment, and the end points themselves. Notably, the concomitant presence of (*a*) tissue injury and (*b*) the potential for uncontrolled hem-

orrhage was outlined as a necessity with any hemorrhage model. Finally, the duration of hypotension before resuscitation and the lethality of the injury should closely mimic clinical situations.

In light of these numerous concerns, we aimed to establish a multicenter animal model of complex combat injury. Our purpose was (*a*) to develop a clinically relevant swine model that could be used to investigate resuscitation after severe multisystem injury and (*b*) reproducibly export this model to other institutions. Specifically, this model was developed for use in an ongoing randomized controlled trial investigating the effects of different ratios of blood products on survival and coagulopathy in swine at three different centers. Our intent was to speed progress in the experiment by using three sites, understanding that the inherent variability of this approach would likely improve the applicability of any finding. To our knowledge, there are no previous reports of a successful multi-institutional animal model for research in hemorrhage and resuscitation.

HYPOTHESIS

An animal model simulating severe complex combat-related injury can be reliably reproduced in a multiple-institution format.

MATERIALS AND METHODS

Investigators at three major medical centers prospectively agreed to use an animal model created at one of the centers. Institutional review board and
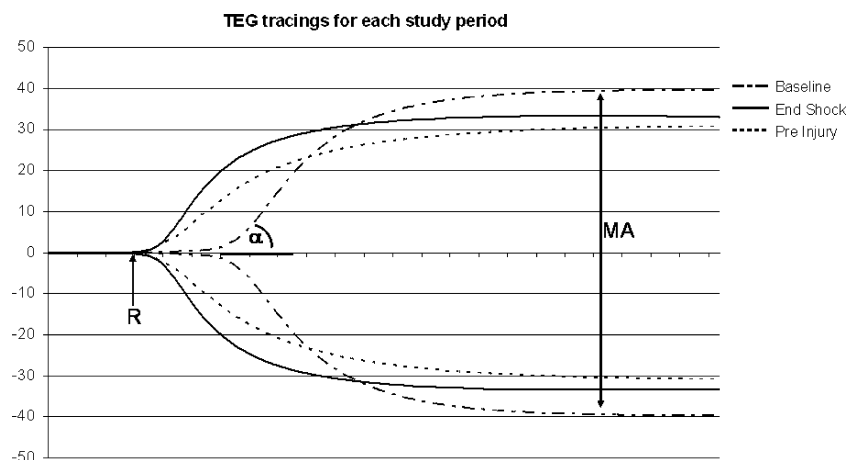
Fig. 2. **Representative TEG tracings from a single study animal**. The *R* time denotes the time until clot formation and reflects activity of soluble coagulation factors. The α angle is the rate of cross-linking and denotes fibrinogen activity. The MA reflects clot strength and is mainly a function of platelets. The superimposed tracings show that at baseline, clot takes longer to form (longer *R* time) but reaches a greater maximum strength (larger MA) at a faster rate (greater a angle). In contrast, at preinjury, clot is initiated sooner but takes a longer time to reach a decreased strength. The end shock tracing is intermediate between the two.

Institutional Animal Care and Use Committee approval was obtained at all three centers. All animals were maintained in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, and all experimental manipulations were performed in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. The model was developed at the Oregon Health and Science University, Portland, Oregon (center 1), and exported to the United States Army Institute of Surgical Research (USAISR, center 2) and Massachusetts General Hospital/Harvard Medical School (MGH center 3).

We developed a complex combat-relevant multisystem injury model of liver injury, long bone fracture and soft tissue injury, and hemorrhagic shock with hypothermia and acidosis. We then simulated an injury phase, a preoperative phase (including prehospital care, transport, and emergency department), and an operative phase of resuscitation. Figure 1 illustrates our model.

Study protocol

Thirty-seven female Yorkshire crossbred swine were used for model development. Animals were delivered 7 to 10 days before the experiment to minimize the stress of transport and subsequent potential changes in sympathetic output or inflammatory mediators. An overnight fasting period was observed with the exception of water *ad libitum*. All animals were ordered such that their weight at the time of the experiment was 39.7 ± 1.1 kg (mean $\pm$ SEM). No attempt was made to use a single vendor, and each center made their own arrangements for procurement of animals according to their standard sources.

Anesthesia—Anesthesia was induced with 8 mg/kg Telazol (tiletamine hydrochloride 50 mg/mL, zolazepam hydrochloride 50 mg/mL, Fort Dodge Animal Health, Fort Dodge, Iowa) i.m. and isoflurane at 1% to 3% inhaled. Orotracheal intubation was performed after which an esophageal thermometer was placed. Throughout the study, anesthesia was maintained to the clinical end points of reflexes and muscle relaxation as is done in humans.

Monitoring, access, and pre-experiment procedures—Vascular access was established via neck cut-down and placement of carotid artery and external and internal jugular vein catheters. The femoral artery was cannulated for blood pressure monitoring. Baseline laboratory specimens were collected and included electrolytes, lactate, spun hematocrit (Hct), platelets, prothrombin time (PT), partial thromboplastin time (PTT), and arterial blood gas (ABG). In addition, a baseline

thromboelastogram (TEG, Haemoscope Corporation, Niles, Ill) was performed. A celiotomy was then performed, at which time a suprapubic bladder catheter was placed to monitor urine output.

Thromboelastography—Thromboelastography is a rapid, functional, point-of-care coagulation test developed in the 1940s that is currently being used to guide decision making in cardiac transplant (23), liver transplant (24), and the combat theater. A fixed amount of blood is placed in a cup into which a pin affixed to a torsion wire is lowered. The cup begins oscillating at a constant amplitude and deflection. As clot begins to form, it adheres to the pin and wire assembly, causing it to deflect. As the clot matures, it causes the deflection to increase, and as thrombolysis occurs, the deflection decreases. A TEG tracing is shown in Figure 2. The *R* value is the time to initial clot formation and represents the activity of the soluble coagulation factors, that is, the *intrinsic and extrinsic pathways*. The α angle represents the rate of cross-linking and primarily reflects fibrinogen activity. The maximum amplitude (MA) is the clot strength and represents platelet activity. Unlike standard clotting assays, TEG is functional, not quantitative, and can be performed at the patient's temperature.

Injury phase—After needle localization, a captive bolt gun (Schermer Stunner Model MKL, Karl Schermer and Co, Karlsruhe, Germany; Fig. 3) was used to fracture the femur and create a soft tissue injury at the midshaft of the left femur. Figure 4 is a three-dimensional computed tomography reconstruction of a typical femur fracture created in a study animal by these methods. A controlled hemorrhage was then initiated at 100 mL/min to remove 60% of the blood volume based on a published standard equation relating blood volume to body weight for domestic swine (25). During this period, if the MAP fell below 25 mmHg, isotonic sodium chloride solution (NS) was infused at a rate of 165 mL/min to keep the MAP higher than 25 mmHg. Typically, the hemorrhage time lasted roughly 20 min, during which NS was infused intermittently for 3 to 5 minutes. The animal was then cooled to $33 \pm 0.4°C$ using cooled intraperitoneal lavage with crystalloid as needed (most of the animals spontaneously developed a degree of hypothermia because of shock and infusion of i.v. fluids). These procedures were followed by a 30-min shock period, representing time in the field before medical intervention.

Prehospital care/transport phase—After the 30-min shock period, electrolytes, spun Hct, PT, PTT, platelets, ABG, and TEG were again recorded. After coagulation studies and laboratory specimen collection, the hemorrhage volume was replaced with a 3:1 ratio of NS infused at a rate of 165 mL/min minus any given during the controlled hemorrhage. This was done to replicate the metabolic acidosis and dilutional coagulopathy of acute trauma. Although military protocols recommend small-volume resuscitation, the use of this fluid regimen results in the desired metabolic derangements while reflecting current civilian prehospital resuscitative practices, maintaining clinical relevance.

Operative phase—After NS resuscitation, a 15-min stabilization period was observed, during which a baseline MAP was recorded, and preweighed laparotomy sponges were placed in both paracolic gutters and in the pelvis for blood collection. Laboratory specimens and coagulation studies were again collected, and a standardized grade V liver injury was created. All procedures up to this point were part of the model development, and it is after this point that randomized interventions such as simulated operative conditions or varying PRBC:FFP ratios could be implemented. We included only those three time points (baseline, post-shock, and preinjury) that were common to all animals so that we could compare the center effect.

Fig. 3. Captive bolt gun used to create femur fracture and soft tissue injury.

FIG. 4. **Three-dimensional computed tomography reconstruction of a typical femur fracture created in a study animal**. The arrow demonstrates a complex left midshaft femur fracture.

Study variables

Physiologic variables included survival, MAP, blood loss from the controlled hemorrhage, and blood loss caused by the liver injury. Laboratory values include Hct, lactate, platelets, ABG, and electrolytes. Coagulation parameters include the PT, PTT, and TEG values.

Statistical analysis

Mean values of study variables between centers were compared using one-way ANOVA. We assumed that our study populations were normally distributed, and that the variances of the populations were equal. A *post hoc* Bonferroni correction was applied to account for multiple comparisons. The significance level was set at a $P < 0.05$. Statistical analysis was performed using SPSS version 15.0 (SPSS Inc, Chicago, Ill).

RESULTS

Three primary time points are reported. Baseline refers to values collected after anesthesia, instrumentation, and laparotomy but before femur fracture (Fig. 1, between steps 2 and 3). End of shock refers to values collected after femur fracture, controlled hemorrhage, hypothermia, and at the end of the 30-min shock period (Fig. 1, between steps 4 and 5). Preinjury refers to values collected after the shock period, resuscitation of NS in a 3:1 ratio, and 15-min stabilization but before the liver injury (Fig. 1, between steps 6 and 7). All means are

reported ± SEM. Eight of the animals died before the end of the model period for a mortality rate of 21.6%. These animals were excluded from the analysis in that all data points could not be collected.

Physiologic variables

Hypothermia was achieved during the shock period, with a pre–liver injury temperature of 33.1°C ± 0.07°C. Blood loss from the controlled hemorrhage, a function of the calculated blood volume, was 1708 ± 35.6 mL or 43.2 ± 0.3 mL/kg body weight.

Mean arterial pressures were not different at baseline ($P = 0.46$) or at the end of shock ($P = 0.37$) between the three centers. Mean preinjury MAPs were not different between centers 1 and 2 ($P = 1.00$) and between centers 2 and 3 ($P = 0.15$), but were different between centers 1 and 3 ($P = 0.046$). Across all centers, there was a 51% drop in blood pressure between baseline and after controlled hemorrhage, femur fracture, soft tissue injury, and hypothermia from a MAP of 70 ± 2.41 mmHg to 32 ± 1.70 mmHg ($P < 0.0001$). Between the end of the shock period and after resuscitation with NS, there was a significant increase in MAP from 32 ± 1.70 mmHg to 46 ± 2.43 mmHg ($P < 0.0001$), an average increase in 14 mmHg but 24 mmHg below baseline. Figure 5 illustrates these results.

Laboratory variables

Baseline, end of shock, and preinjury spun Hct values were similar between centers ($P > 0.10$). A significant decrease in Hct was seen over the three study phases from a mean of 28.7 ± 0.58 at baseline, to 22.3 ± 0.86 at the end of shock, to 15.3 ± 0.65 at preinjury. The change in Hct between baseline and the end of shock was significant ($P < 0.0001$), as was the change in Hct from the end of shock to preinjury ($P < 0.0001$). Figure 6 illustrates these results.

There was no difference between arterial pH values at baseline, end of shock, and preinjury ($P > 0.10$) between centers. Mean overall pH decreased from 7.453 ± 0.01 at baseline, to 7.384 ± 0.01 at the end of shock, to 7.289 ± 0.02 at preinjury. The pH decreased significantly between each time point ($P < 0.001$). The pco_2 values were not different between centers at each time point ($P > 0.05$). Mean pco_2 was 43.1 ± 1.36 mmHg at baseline, 37.3 ± 1.95 mmHg at the end of shock, and 37.1 ± 1.79 mmHg at preinjury. The pco_2 dropped
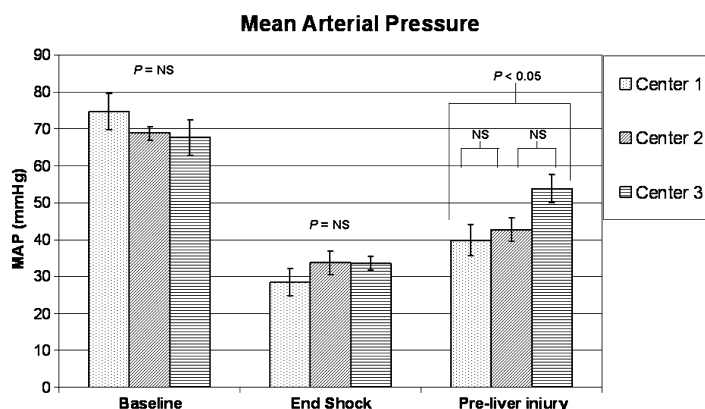

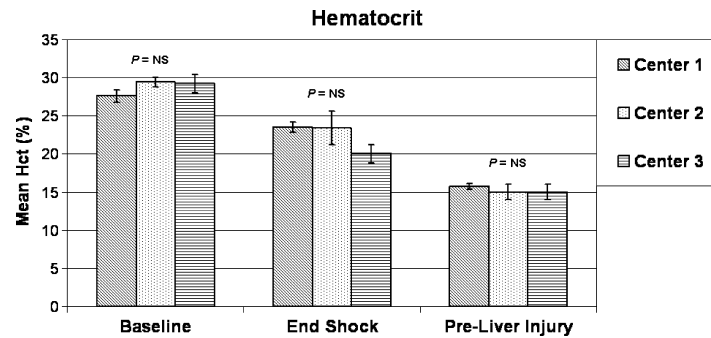FIG. 5. **MAP at the three different time points**. All values are mean ± SEM. NS–nonsignificant.

FIG. 6. **Hematocrit (Hct) at the three different time points**. All values are mean SEM. NS–Nonsignificant.

7.1 ± 1.92 mmHg between baseline and the end of shock ($P < 0.01$) but did not change significantly between the end of shock and preinjury, during which time the only intervention was resuscitation with NS. Figure 7 illustrates these results.

Lactate and base deficit (BD) were different among the three centers at baseline and the end of shock periods. By pre–liver injury, after resuscitation with NS, all BD and lactate values were similar among the three centers. At preinjury, the mean BD was 8.2 ± 0.65 mEq/L, and the mean lactate was 5.3 ± 0.44 mmol/L. The BD increased at each center between baseline and preinjury ($P < 0.0001$). Lactate values increased at each center from baseline to preinjury ($P < 0.0001$). Figure 8 illustrates these results.

After femur fracture, induction of hypothermia, and 60% controlled hemorrhage, there was a corresponding 51% drop in the MAP from baseline. Resuscitation with NS increased the blood pressure partially but below baseline. The Hct dropped through all three of these stages, corresponding first to injury and hemorrhage, then to dilution before the liver injury. Metabolic acidosis is demonstrated by a progressive drop in pH, whereas the pco_2 remains relatively constant. Although different among centers at baseline and the end of shock, the BD and lactate values were similar at preinjury and had mean values of 8.2 ± 0.65 mmol/L and 5.3 ± 0.44 mmol/L, respectively, further demonstrating the metabolic component of the acidosis. Figure 9 illustrates these relationships.

Coagulation parameters

The PT and PTT differed significantly between the three centers, as is demonstrated in Tables 1 and 2. Although

different at each center, within each center, the PT increased significantly over time ($P < 0.05$), indicating the development of a progressive coagulopathy. The PTT value did not increase over time ($P > 0.05$), indicating that the coagulopathy was not reflected by measurement of this parameter.

The mean R value by TEG, was similar between all three centers for baseline and the end of shock. However, by pre–liver injury, center 3 differed from centers 1 and 2, whereas centers 1 and 2 were similar. Normal values for R time are between 4 and 8 min. This is the time to initial clot formation in patients with normal coagulation function. Animals at all three centers had normal mean values at baseline, with minimally hypercoagulable values at the end of shock. Centers 1 and 2 also had minimally hypercoagulable or normal mean values at preinjury, whereas center 3, which differed significantly from centers 1 and 2, had mean hypercoagulable values. Table 3 illustrates these results. Although values differed between the centers, the trends were similar.

The α angle, representing the rate of fibrinogen cross-linking, was similar at baseline and the end shock among all three centers. By ANOVA, there was a significant difference between centers by pre–liver injury. When the Bonferroni correction was applied for multiple comparisons, there was a trend toward a significant difference between centers 1 and 2 at this time point ($P = 0.08$), but no significant differences. Over time, the α angle did not significantly change, indicating a constant rate of fibrinogen cross-linking over the model period ($P = 0.34$). The MA, reflecting the strength of platelet interaction, was similar among the three centers. Over time, the MA decreased significantly at each successive time point
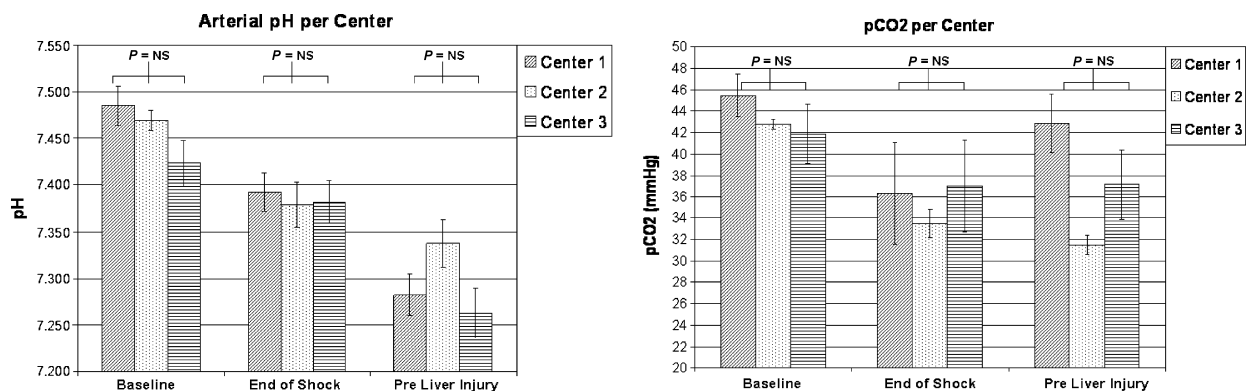

FIG. 7. **Arterial pH and P_{CO2} at the three time points**. All values are mean $\pm$ SEM. NS–nonsignificant.
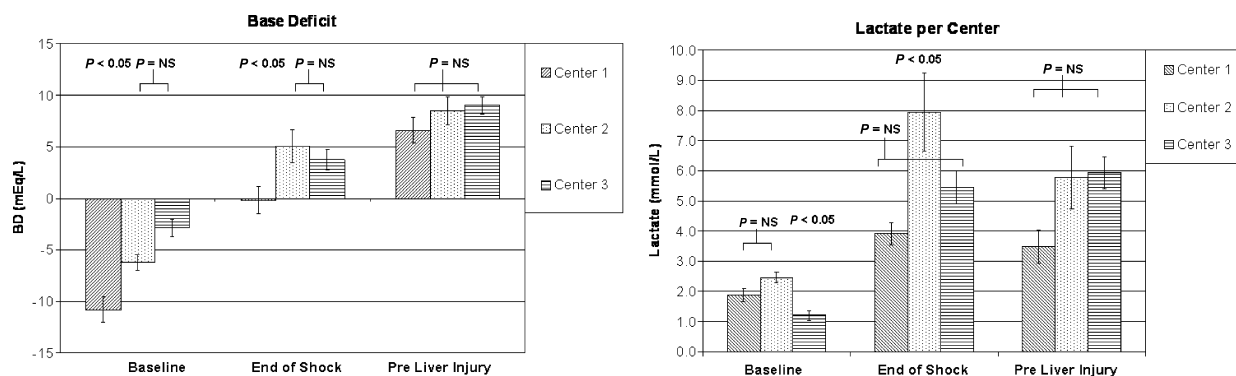
Fig. 8. **Lactate and BD at the three time points**. All values are mean ± SEM. NS–nonsignificant.

after baseline, indicating progressively weaker platelet activity ($P < 0.001$). Tables 4 and 5 illustrate these results.

DISCUSSION

We developed a new swine model to create a multisystem injury with both controlled and uncontrolled hemorrhage components, and with the potential for rebleeding. We also induced hypothermia, coagulopathy, and acidosis to introduce the *lethal triad* of trauma. In addition, we simulated three distinct components of patient care: (*a*) an injury phase, (*b*) a prehospital or transport phase, and (*c*) an operative phase. It is at this point, during a potential fourth treatment phase, that specific interventions can be applied, such as different resuscitation fluids and protocols, hemostatic dressings, monitoring or surgical devices, hemoglobin carriers, and operative techniques, to name a few. This model is highly clinically relevant in its replication of both the physiology of severe trauma and the phases of patient care.

Physiologic parameters

Physiologic parameters and laboratory values correlate logically and plausibly with the simulated clinical phases during the course of the study. Injury and controlled hemorrhage caused a more than 50% decrease in the MAP, whereas resuscitation with NS increased the blood pressure. The Hct dropped predictably after injury and controlled hemorrhage, and dropped further after dilution with NS. Correspondingly, the drop in pH and relatively constant pco_2 demonstrate a metabolic acidosis, as would be expected. In addition, this indicates that the animals were ventilated consistently across all centers. Although there was a significant difference among centers in the starting BD and lactate, there was also a statistically significant increase in BD and lactate over time, which was seen at all three centers. By preinjury, all centers had similar BD and lactate values, with a mean (±SEM) overall BD of 8.2 (± 0.65) mEq/L and mean overall lactate value of 5.3 (± 0.44) mmol/L.

The overall mortality rate of 21%, BD, and lactate values by pre–liver injury support the severity of the model. Elevated BD and lactate levels are independent predictors of mortality in critically ill patients (26, 27). Kaplan and Kellum (26) reported that 98% of nonsurvivors with major vascular injury had a

lactate level greater than 5 mmol/L and a BD of 7.3 mEq/L (compared with our animals' mean lactate level of 5.3 mmol/L and BD of 8.2 mEq/L). Figure 9 demonstrates that the mean BD at baseline is −6.0 ± 0.82 mEq/L. This baseline alkalosis has been observed in prior swine studies and may reflect the inherent physiology of the species. The absolute value of the BD preinjury may underestimate the severity of the systemic insult. The mean change in BD in our animals was 14.2 mEq/L, reflecting a very severe model.

Krishna and colleagues (27) reported that the combination of a BD greater than 5 mEq/L and temperature less than 35.5°C was an independent predictor of death in patients with multiple visceral injuries who underwent operations, which closely resembles the conditions of our model (our mean temperature was 33.1°C ± 0.07°C). Notably, each parameter exhibits a small SEM, supporting the excellent reproducibility of this severe model. The similarities in our results indicate that given a similar complex of injury and metabolic insult, a similar physiologic profile (dilution, acidosis, and hypothermia) can be reliably achieved and measured in a multiple-institution format.

Coagulation parameters

Coagulation parameters did not exhibit the same degree of uniformity. All three centers differed significantly from each other at baseline and preinjury for PT, whereas centers 1 and 2 were similar to each other and different from center 3 at the end of shock. Centers 1 and 2 were similar to each other but different from center 3 at all time points for PTT. One trend that makes itself apparent is that the PT steadily increases over the model period, indicating a progressive coagulopathy. The PTT value did not reflect this change. *R* values were similar between centers at baseline and at the end of shock, but by preinjury, centers 1 and 2 were similar and different from center 3. Many factors could account for this variability, including intrinsic characteristics of the animals, differences in blood loss or fluid administration, time to measurement, differences in reagent, calibration and type of machine used to measure values, and operator error. Given the similarity in physiologic variables, it is likely that factors related to the animals and conduct of the experiment contribute less to this variation than differences in measurement and machine factors. This is particularly the case given
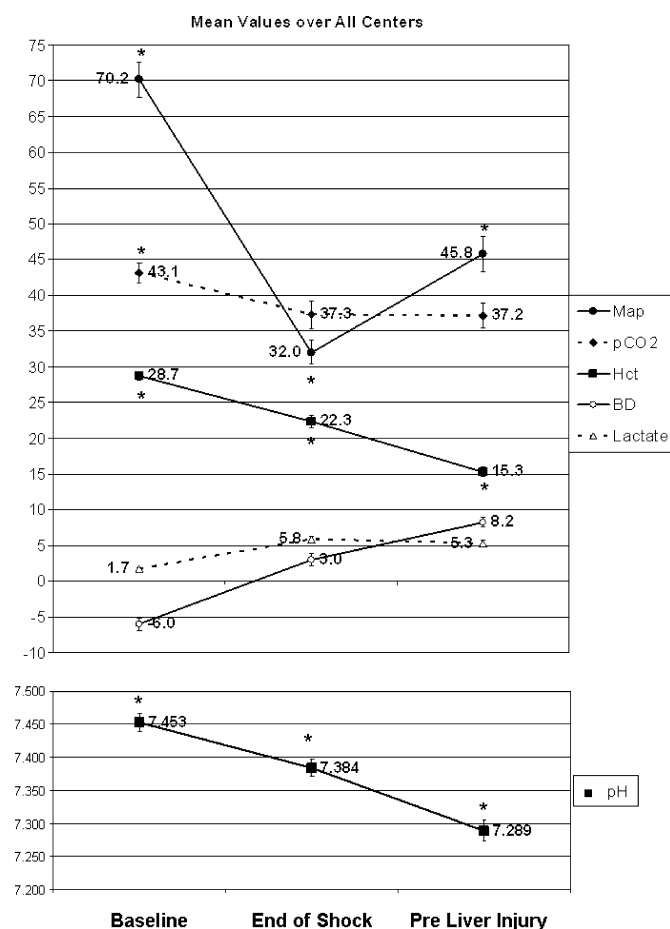
FIG. 9. **Mean values averaged over all three centers for each of the study time points**. All values are mean ± SEM. Asterisks (*) denote values significantly different (*P* < 0.05) from the other time points.

TABLE 1. PT values between centers

PT, s	Center 1	Center 2	Center 3	P
Baseline	13.0 ± 0.2	10.5 ± 0.1	20.5 ± 0.6	<0.001
End of shock	14.4 ± 0.4*	12.3 ± 0.7*	22.9 ± 1.1†	<0.001
Pre–liver injury	20.0 ± 1.5	15.1 ± 0.8	24.5 ± 1.1	<0.001

Values are expressed as mean ± SEM.
*No significant difference between centers 1 and 2 at this time point.
†Center 3 differed from both centers 1 and 2 at this time point.

but were not statistically different than baseline. These findings are similar to our results as outlined in Tables 1 and 2. Yuan and colleagues (29) determined that a dilution of any one of the vitamin K–dependent factors below 35 IU/dL resulted in a PT/international normalized ratio (INR) of greater than 1.5 times normal. They also found that the PT/INR correctly identified a vitamin K–dependent factor deficiency in trauma patients 84% of the time. Similarly, a dilution of the factors of the classical intrinsic pathway to the same level caused an elevation of the PTT to greater than 1.5 times normal. However, the PTT correctly identified a factor deficiency only 50% of the time in trauma patients. They propose that this may be caused by an elevation of factor VIII as an acute-phase reactant, which can falsely normalize the PTT while leaving the PT elevated.

Alteration of coagulation demonstrated by TEG has been seen in other animal studies. For example, Kiraly and co-workers (20) noted a decrease in *R* value (hypercoagulability) over 2 h in a grade V liver injury swine model comparing NS to LR resuscitation. They noted a significant difference between LR and NS (LR leading more toward hypercoagulability), and a significant difference between baseline and 60-, 90-, and 120-min values. Their changes were statistically significant but within the reference range, similar to our findings in that our decreases in *R* value were either within the reference range or minimally outside of it. A reduction in *R* values was also reported by Nielsen and colleagues (30), who performed a series of *in vitro* experiments on serially diluted plasma. They found a decrease in the *R* time in plasma diluted up to 30%, from a baseline of 705 to 594 s. Furthermore, the *R* value was diminished but remained unchanged with serial dilutions in plasma deficient in antithrombin, leading them to conclude that a preferential washout of antithrombin is central to this dilutional decrease in the *R* time. This phenomenon has also been observed in the *in vivo* setting, as reported by Ng and coworkers (31). These authors randomized 20 patients to either controlled 30% hemodilution with NS before elective hepatobiliary surgery versus no intervention. They performed serial TEGs at 10-min intervals and found a 30% decrease in the

evidence questioning the reliability of PT and PTT measurements. Traditional clotting assays are notoriously variable across centers and between assay methods (28). Specifically, centers 1 and 2 used the Start-4 coagulation analyzer (Diagnostica Stago, Asnières sur Seine, France), whereas center 3 used the SCA-2000 coagulation analyzer (Delta Scientific Inc, Ivyland, Pa). This likely contributes to the similarity in PT and PTT between centers 1 and 2, whereas the values from center 3 differed. Based on the variability in some of the coagulation assay measurements, we elected to centralize these measurements at center 2 during the ongoing multi-institutional preclinical study, which has minimized this variability.

Coagulation abnormalities

The progressive increase in the PT value has been described in trauma patients. For example, Ledgerwood and Lucas (5) demonstrated elevations in the PT for up to 15 h postoperatively in 22 trauma patients undergoing operations and requiring an average of 21 units of blood. Furthermore, factor V levels fell intraoperatively and remained low during this period, with a nadir of 43% of normal and a value of 77% of normal at 15 h. However, the PTT values remained within reference range during the first 4 postoperative days and at hospital day 25. Factor VIII levels dropped to 63% of normal in the operating room, but increased and remained elevated. During the first 15 h, levels were 111% to 127% of normal,

TABLE 2. PTT values between centers

PTT, s	Center 1	Center 2	Center 3	P
Baseline	25.2 ± 3.7*	15.3 ± 0.2*	55.0 ± 3.1†	<0.001
End of shock	21.8 ± 2.6*	16.7 ± 0.4*	55.8 ± 4.5†	<0.001
Pre–liver injury	22.0 ± 1.3*	19.1 ± 0.5*	58.3 ± 5.4†	<0.001

Values are expressed as mean ± SEM.
*No significant difference between centers 1 and 2 at this time point.
†Center 3 differed from both centers 1 and 2 at this time point.

TABLE 3. ***R* values between centers**

R, min*	Center 1	Center 2	Center 3	*P*
Baseline	5.6 ± 1.0	6.2 ± 0.5	5.6 ± 2.2	0.92
End of shock	3.5 ± 0.2	3.9 ± 0.5	3.9 ± 1.0	0.91
Pre–liver injury	3.8 ± 0.3[†]	4.8 ± 0.2[†]	2.2 ± 0.4[‡]	<0.001

Values are expressed as mean ± SEM.
*Reference range, 4 to 8 min.
[†]No significant difference between centers 1 and 2 at this time point.
[‡]Center 3 differed from both centers 1 and 2 at this time point.

TABLE 4. ***α* Angle values between centers**

α Angle, degrees*	Center 1	Center 2	Center 3	*P*
Baseline	66.6 ± 4.2	69.1 ± 1.5	68.7 ± 5.4	0.88
End of shock	73.7 ± 1.8	73.9 ± 1.4	65.8 ± 4.9	0.14
Pre–liver injury	73.5 ± 1.4[†]	68.0 ± 1.3[†]	73.2 ± 2.3	0.05[‡]

Values are expressed as mean ± SEM.
*Reference range, 47 to 74 degrees.
[†]$P = 0.08$ between centers 1 and 2 by *post hoc* analysis.
[‡]$P = 0.05$ between centers by ANOVA.

R time at 30 min but not at other time points versus control. Although they also saw a decrease in antithrombin levels, they were not able to report this as the definitive cause of the decrease in *R* time.

It is not immediately evident why the *R* value would decrease over the study period, as seen in other studies, whereas the PT would increase. However, the PT is derived from spun plasma that is devoid of cellular elements, and the test is performed at 37°C. The TEG is performed on whole blood at the animal's body temperature. Schreiber and colleagues (32) performed daily TEGs on 65 trauma patients over the first 4 days of their hospital course. They found no correlation between the INR and the *R* value, with the Pearson correlation coefficients ranging between -0.10 and 0.10. They did find significantly elevated INR values on days 1 and 2 compared with days 3 and 4, but these differences were within the reference range (1.11–1.13 for days 1 and 2 vs. 1.06–1.07 for days 3 and 4). Furthermore, they reported normal PTT values that did not change over the study course and shortening of the *R* value on TEG early after injury in most patients.

The *α* angle did not change over the study period, reflecting a constant rate of fibrinogen cross-linking over time. Nielsen et al. (30) also found that the *α* angle remained unchanged in plasma diluted by 30%, whereas it decreased in plasma diluted 40% to 50% from a baseline of 63.1 degrees to 46.2 degrees. Kiraly et al. (20) found a modest increase in the *α* angle with LR resuscitation in their grade V liver injury swine model but no change with NS resuscitation. Our results are similar to those of these two groups, who found a potential increase in coagulability with dilution. Martini et al. (33) noted that acidosis decreases the *α* angle, and also noted that the rate of fibrinogen synthesis is unchanged, although the rate of breakdown is increased. These results were corroborated by Engstrom and colleagues (34), who noted a very strong linear correlation between pH and the *α* angle using rotational TEG. However, the pH studied by Martini et al. (33) was significantly lower than the preinjury pH documented in the current study (7.1 vs. 7.29). Furthermore, Engstrom et al. (34) note a clinically negligible decrease in the *α* angle from normal at this pH, a difference of approximately 3 to 5 degrees and still within the reference range, which is consistent with our results. In another report, Martini et al. (33) also note that hypothermia to 32°C decreased fibrinogen synthesis by 50% but did not affect breakdown. This author also notes that although hypothermia decreases fibrinogen synthesis, a clinical manifestation may be delayed because of a very slow rate of synthesis compared with pool size. The example presented is a 50% decrease in fibrinogen synthesis, with no change in

breakdown, which results in a detectable change 6 h after the insult, which is beyond our study period.

In contrast, the MA value, which was similar among all centers at all time points, decreased significantly over the model period, indicating progressively weaker platelet activity over time. Other reports have corroborated these findings in animal models of hemorrhage (5, 20), which is plausible given the loss of both platelet mass with blood loss and factors responsible for decreased aggregation and adhesion with dilution and hypothermia.

Kheirabadi and coworkers (35) demonstrated the use of TEG for the assessment of coagulation in a rabbit hemorrhage model. The TEGs were performed on three groups of animals, a control group, a group made coagulopathic by administration of warfarin, and a group that was cooled, underwent laparotomy, splenic injury, and resuscitation with a hetastarch-based crystalloid. There was no difference in PT between the normal and hypothermic hemodiluted groups. However, TEG revealed changes in clot formation rate and clot strength. Clearly, the combined effects of dilution, acidosis, and hypothermia on coagulation are complex, as is evidenced by the growing body of literature on this topic. There are considerable research efforts currently underway designed to better characterize coagulation after trauma.

A recent review summarizing current ideas on animal models for hemorrhage and resuscitation research (22) outlined important variables in model design and summarized the recommendations of the 2000 Military Medicine Workshop on Animals Models in Hemorrhage and Resuscitation research. This meeting brought together researchers from the United States Navy, the United States Army, the United States Air Force, the UK Ministry of Defense, and the Swedish Defense Research Establishment. Some of the key needs that were identified at that meeting were (*a*) the need for volume-controlled models that had the potential for compensation and decompensation, (*b*) surgical manipulation coincident with hemorrhage as in clinical situations, (*c*) significant soft tissue injury to better approximate the postinjury inflammatory state, (*d*) a degree of hypotension demonstrated clinically to lead to poor outcomes,

TABLE 5. **MA values between centers**

MA*	Center 1	Center 2	Center 3	*P*
Baseline	78.4 ± 1.9	76.2 ± 1.5	75.8 ± 2.5	0.59
End of shock	70.5 ± 2.9	68.8 ± 2.1	71.9 ± 4.0	0.78
Pre–liver injury	66.1 ± 2.8	59.3 ± 2.3	63.3 ± 2.0	0.37

Values are expressed as mean ± SEM.
*Reference range, 55 to 73 mm.

and (*e*) a model simulating battlefield trauma rather than one designed to meet a specific scientific goal.

We believe that we have addressed these issues in our model. Specifically, points *a* to *c* are addressed by femur fracture, soft tissue injury, and laparotomy. The degree of hypotension demonstrated in Figures 5 and 9 addresses point *d*, whereas controlled hemorrhage, hypothermia, and acidosis with NS resuscitation contribute to the clinical relevance of the model (point *e*). We have also identified several key potential areas for variability. They are listed in Table 6. The reproducibility of our laboratory and physiologic data before the liver injury support the overall uniformity of our model without sacrificing real-world conditions. That is, any potential variability caused by these factors is demonstrably neutralized, as evidenced by data rather than speculation.

Although our lactate and BD values started out significantly different between centers, there were no differences before the liver injury. Investigators could then be reassured that not only is a clinically relevant, multisystem, severe injury state achieved, but that it is the same starting point from which to compare interventions.

Similar large animal models have contributed significantly to the current understanding of optimizing resuscitation. For example, Todd and coworkers (36) noted decreased fluid requirement and decreased hypercoagulability in a grade V liver injury swine model in which animals were randomized to resuscitation with either Hextend (Biotime, Berkeley, Calif) or LR. They also noted no difference in coagulation between LR and control swine, which received no resuscitation. Both groups were hypercoagulable compared with the Hextend group. Watters et al. (37) randomized swine using a similar uncontrolled hemorrhage model to resuscitation with either LR or NS and showed an increased need for fluid in the NS group and no difference in inflammatory mediators (IL-6, TNF-α, and granulocyte colony-stimulating factor). Schreiber et al. (38) reported an early experience with recombinant factor VIIa in hypothermic dilutionally coagulopathic swine with grade V liver injuries randomized to either high- or low-dose treatment drug or control. They found increased factor VII activity, decreased blood loss, decreased MAP, decreased PT, and equivalent survival. There was no evidence of microthrombosis on postmortem lung histology, and there was no difference in hemostatic effect between the two treatment doses. In a swine liver injury model, Pusateri and colleagues (39) found a decrease in PT and TEG parameters with increasing doses of recombinant factor VIIa up to a dose of 90 μg/kg, after which increasing doses had no effect on coagulation. Furthermore, they found no histological evidence of

TABLE 6. **Potential for variability in developing animal models**

Animal differences and source

Availability and experience of personnel

Conduct of experiment

Laboratory environment

Laboratory testing variability

Equipment variability

intravascular coagulation after tissue harvest. Sondeen et al. (40) reported the first evidence that recombinant factor VIIa increased the blood pressure at which rebleeding occurs and decreased the consequent hemorrhage over controls in a swine aortotomy model. Other studies have shown that fluid resuscitation of any kind increases the transcription of inflammatory genes (19) and that 3% or 7.5% hypertonic saline–containing solutions may be superior to large-volume resuscitation in similar models (18). Work such as this has led to the recommendation of resuscitation with a 6% heta-starch in a balanced crystalloid carrier (Hextend) in the battle-field (41, 42).

LIMITATIONS

Our study had several limitations. Most notable was the significant difference in some of the coagulation parameters. As previously mentioned, we feel that this more likely reflects the use of different equipment and assays than actual differences between centers given the similarity of other physiologic parameters such as degree of acidosis, Hct, and MAP. We have now centralized the coagulation testing at one center, which has decreased this variability. Another consideration is that of hypotensive resuscitation. That is, during our prehospital phase, we administered a volume-triggered amount of NS in a 3:1 ratio of the controlled hemorrhage volume. This does not necessarily reflect a current practice, which is to resuscitate to maintain critical end-organ perfusion (e.g., mental status) (39), which is not feasible in an animal model. However, one of the purposes of the NS resuscitation was to contribute to the acidosis of shock and to the dilutional coagulopathy, which was achieved with uniformity across all centers. This adds to the clinical relevance and severity of the model and therefore is acceptable.

CONCLUSIONS

We aimed to establish an animal model simulating complex combat-related injury. This model was reliably reproduced in a multiple-institution format. Acidosis, coagulopathy, and hypothermia were introduced, increasing clinical relevance. The degree of metabolic acidosis development, Hct drop, and MAP decrease were similar across all centers and reflected the different simulated phases of our model. These included an injury phase, a prehospital and transport phase, an operative phase, and an intensive care unit phase. Although coagulation parameters differed, this was likely caused by differences in technology and will be addressed by centralizing the testing. We have shown that a complex combat-related model can be developed in a single center and exported to two other centers with excellent reproducibility. This should permit more rapid testing of resuscitation protocols and hemostatic agents and ensure that results can be generalized to multiple environments.

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